COMPARATIVE STUDIES OF THE PIGMENTS OF SOME PACIFIC COAST ECHINODERMS [1]

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From the standpoint of comparative biochemistry, the echinoderms represent an interesting and little-explored phylum. Prominent among the biochemical features of this group is the conspicuous manifestation of body-pigments, striking in their intensity and color-varieties, and rivalling the beauty of those displayed by the sessile coelenterates.

The integumentary colors of sea-stars and brittle stars are due preponderantly to carotenoids, while certain of the echinoids manifest instead considerable quantities of pigments of the echinochrome class, first reported by MacMunn (1883*a*) and recently shown by Kuhn and Wallenfels (1939) to be naphthoquinones. Kuroda and Ohshima (1940) have crystallized three distinct spinochromes, each from a different species of Japanese sea-urchin, and have found the natural pigments to be very similar to synthetic hydroxynaphthoquinones. Some echinoids yield echinenone, a unique carotenoid which is a provitamin A (Lederer and Moore, 1936). Long ago, MacMunn (1883*b*, 1886) reported the presence of " enterochlorophyll " in the alimentary organs of a number of carnivorous echinoderms.

Studies of Abeloos (1926) and Lönnberg (1931, 1932, 1933) give qualitative indications of the nature and distribution of carotenoids in echinoderms. Euler and Hellström (1934) and Euler, Hellström and Klussman (1934) made chemical studies of the carotenoid proteins of asteroids, and isolated a new pigment, asteric acid. Karrer and Benz (1934) and Karrer and Solmssen (1935) isolated astacene from both an ophiuran and an asteroid. Lederer (1938) has studied the pigments of the echinoid *Strongylocentrotus lividus*.

Numerous writers emphasize the importance of the question as to whether some of the lower animals may be able to synthesize specific carotenoids from simpler molecules. Among the various ecological factors and physiological activities which may influence the pigmentation of animals, it is probable that food exerts the closest and most direct effect, although habitat and various physiological adaptations inseparably

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associated with nutrition are necessarily important in a survey such as we have undertaken.

Of the rather scanty information available regarding the physiology of adult echinoderms, facts concerning their feeding mechanisms and digestive enzymes are probably the chief entries. Yonge (1928) discusses the diversity of feeding mechanisms employed by the Echinodermata, and divides the phylum into two main groups as regards feeding and digestion, namely: (1) the Asteroidea and Ophiuroidea which are exclusively carnivorous, and (2) the Echinoidea and Holothuroidea, chiefly herbivorous and to some extent omnivorous. He suggests (1931) that the ciliary-feeding Crinoidea may be primarily herbivorous.

In the present study, we have attempted to make a preliminary classification of the various echinoderms into biochemical (pigment) types, with correlative differences in intraphylar class, sex (where practicable), habitat and nutritional habits occupying collateral positions of importance.

The survey, which included four species of three genera of echinoids, one species of holothuroid, four species of three genera of asteroids, and three species of two genera of ophiuroids, revealed numerous familiar and a few new carotenoids. Purple echinochrome pigments were observed only in echinoids. Small amounts of green " enterochlorophylls " were found among the asteroids and ophiurans. We have considered the echinoderm classes in ascending evolutionary series within the two chief nutritional groups named by Yonge (1931) i.e. (1) herbivore-omnivores: (a) echinoids, (b) holothuroids; and (2) carnivores: (a) asteroids, (b) ophiurans. Consistencies in this classification will be apparent in Table I.

The animals were collected at several localities in this vicinity of the Southern California coast, and in some cases obtained by dredge hauls. We are indebted to Mr. P. S. Barnhart, curator, Dr. C. E. Moritz, visitor from Dartmouth College, Professor G. E. MacGinitie of the California Institute of Technology, Mr. Granville Ashcraft of the Hancock Foundation, University of Southern California, Professor H. J. van Cleave, visitor from the University of Illinois, and Mr. L. D. Pratt of the Kelco Company, National City, for much of the material used. Mr. Sheldon C. Crane rendered technical assistance during the latter part of our carotenoid investigations.

Methods

With but few exceptions noted below, the animals were placed temporarily in running sea water in laboratory aquaria, to permit foreign material to be evacuated from the intestine. The carotenoid pigments were extracted from the ground tissues with acetone, passed into petroleum ether, subjected to partition between the latter and 90 per cent methanol, and separated into individual components by chromatographic adsorption, in accordance with regular procedures described in more detail by Scheer (1940) and by Fox and Pantin (1941).

Individual pigments were provisionally identified by comparison of certain of their properties with those of known pigments; this identification must wait upon further studies for full confirmation. The following properties were used: (1) Behavior in partition between immiscible solvents, i.e., petroleum ether (which constitutes the epiphase and dissolves carotenes, xanthophyll esters and a few monohydroxyxanthophylls) and 90 per cent methanol (the hypophase which selectively removes xanthophylls). (2) Adsorption behavior on Tswett chromatographic columns, xanthrophylls and their common esters being adsorbed from benzene solution on calcium carbonate, while carotenes pass through this but are selectively adsorbed on calcium hydroxide (Zechmeister and Cholnoky, 1937; Strain, 1938). (3) Positions of spectral absorption maxima. (4) Behavior toward the partition test after treatment with hot alcoholic potassium hydroxide in an inert atmosphere; carotenes remain unchanged; xanthophylls, epiphasic when esterified, are rendered hypophasic when hydrolyzed; astaxanthin, free or esterified, neutral before treatment, is transformed into astacene, with definite acidic properties.

Positions of absorption maxima were determined with a Hartridge reversion spectroscope and with a Bausch and Lomb spectrophotometer. The two instruments show good agreement excepting in the case of acidic carotenoids like astacene, whose single broad maximum is more accurately determined with the latter instrument. Carbon disulphide was employed as the solvent unless otherwise specified.

Relative concentration of mixed carotenoid pigments (i.e., whole epiphasic or whole hypophasic fractions) were estimated in terms of "β-carotene equivalents," the extinction coefficient of β-carotene at 485 mμ being determined by other writers (Smith, 1936) and ourselves.

Echinochrome pigments were readily extractable from the echinoids by treatment of the whole tests with dilute hydrochloric acid under a layer of diethyl ether which readily dissolved the pigments (see Tyler, 1939). These were subsequently examined spectroscopically in ether or chloroform solutions.

Green pigments ("enterochlorophylls") were recovered in small amounts from the digestive diverticula of two of the asteroids, *Pisaster ochraceous* and *P. giganteus,* and from the whole-body extracts of all three ophiurans. The material recovered from the ophiurans differed in certain solubility properties from that yielded by the asteroids, but all showed closely agreeing absorption spectra. Actual quantities were so small that no identifying tests were practicable excepting those employed; hence the descriptive term coined by MacMunn has been used provisionally in the discussion of these green pigments.

Results

A summary of the distribution of carotenoids, echinochromes and enterochlorophylls is given in Table I, followed by more detailed information regarding the separate pigments.

Carotenoids

The distribution, kinds, and some relative quantities of individual carotenoids encountered in the various species are summarized in Table II.

Echinochromes

These pigments, present exclusively in the echinoids, showed a few interesting variations in tissue distribution as well as in chemical and spectroscopic properties, as shown in the following outline:

Dendraster excentricus: Purple aggregates of the pigment were present in ectodermal and endodermal tissues lining the shell of this purple sand-dollar, while similar bodies in mature male and female gonad tissues and in anterior portions of the gut were red. The posterior part of the gut lacked echinochrome. The gelatinous egg-cases contained red echinochrome bodies.

The pigment was purple in neutral or alkaline media and red in acid (see also Lederer, 1940). It was readily soluble in aqueous acetone, giving clear filterable solutions, and was also extractable from dilute acid digests of the shell with diethyl ether (see Tyler, 1939).

The absorption maxima were as follows:

In acetone: 524, 490 mμ.

In chloroform: 533, 496, 465 mμ (*cf.* Kuhn and Wallenfels, 1939).

Strongylocentrotus franciscanus: Much purple pigment was yielded to acetone by treatment of this large purple-red urchin. The pigment decomposed with bleaching, however, before it was given any study.

Strongylocentrotus purpuratus: This purple urchin, like the sand-dollar *Dendraster,* showed many aggregates of echinochrome in the ectoderm (purple) as well as in endoderm, coelomic fluid and gut wall (red in all). Neither male nor female gonad tissues, however, contained any of the pigment.

It showed the following absorption maxima:

In water (neutral, colloidal): 526.5, 591 mμ.

In water (acidic): 497 mμ (single, diffuse band).

In chloroform: 525, 490.5 mμ (*cf.* Lederer and Glaser, 1938).

Lytechinus pictus: This pale urchin yielded far less of the purple pigment than did *Strongylocentrotus* or even *Dendraster.* Pigment aggregates were observable, however, in parts of the skin lining the shell, in the gut and in gonad tissues of both sexes.

The pigment, insoluble in acetone or water, readily bleached by alkali, and slightly soluble in dilute acid, gave the following spectral absorption bands:

In water (colloidal suspension): 526, 491 mμ.

In diethyl ether: 533, 497, 467 mμ.

The echinoids contained not only different quantities of echinochrome pigments, in the order *Strongylocentrotus* > *Dendraster* > *Lytechinus,* but the pigments were somewhat different chemically. Lederer (1940) lists the distribution of a number of pigments of the echinochrome class in several echinoid species, and in their separate body parts.

Green Pigments

The "enterochlorophylls," found only in the digestive diverticula of two of the five species of asteroids and in whole extracts of the three ophiuroids, showed certain chemical differences.

Asteroidea

Pisaster ochraceous: A small quantity of the pigment was recovered in acetone from the digestive diverticulum, being absent from stomach, skin or other parts. Completely insoluble in petroleum ether, it was readily soluble in alcohol, acetone and diethyl ether. Its non-acidic character was demonstrated by its extractability from diluted alkaline alcohol with ether. Dissolved in absolute ethanol, it manifested a single sharp absorption band in the red at 661 mμ.

Pisaster giganteus: The green pigment in this species, recovered in traces from the same tissues as in *P. ochraceous,* was similar in its chemical properties and also yielded a single absorption band in pure ethanol at 661 mμ.

Astropecten californicus and *Patiria miniata* failed to yield green pigments.

Ophiuroidea

Ophiopteris papillosa: Extracted with acetone and soluble also in diethyl ether, the pigment was dissolved readily in petroleum ether (un-

TABLE I

Summary of Pigments in Echinoderms Studied

Animal (number analyzed)	Tissue; weight grams	Total epiphasic carotenoids mg./100 g.	Total hypophasic carotenoids mg./100 g.	Ratio; hypophasic/epiphasic	Carotenes	Xanthophylls	Xanthophyll esters	Acidic carotenoids or their precursors	Predominating *types* of carotenoid	Echinochromes	"Enterochlorophylls"
HERBIVORES; OMNIVORES											
ECHINOIDEA											
Dendraster excentricus											
(3 ♀)	whole; 31	0.052	0.011	0.211	++	+	–	...	β-carotene	+	–
(2 ♂)	whole; 20	0.105	0.038	0.362							
Strongylocentrotus franciscanus											
(1 ♂)	whole; 298	0.170	0.025	0.147	++*	+	–	...	β-carotene	+	–
S. purpuratus											
(3 ♀)	skin ...	traces	traces	...			± (varying)	–	zeaxanthin, β-carotene.	+	–
	intestine; 5.67	2.99	6.04	2.02	++*	++					
	gonad; 8.64	2.00	0	0	++*	–					
(3 ♂)	skin ...	traces	traces	...							
	intestine; 4.72	2.34	5.65	2.41	++*	++					
	gonad; 11.9	0.690	0	0	++*	–					
Lytechinus pictus											
(26 ♀)	whole; 131	0.493	0.351	0.712	++*	+	–	...	α- and β-carotenes, echinenone.	+	–
(13 ♂)	whole; 67	0.871	0.430	0.493							
(9 ♀)	intestine; 0.9	4.48	4.18	0.934	++	+					
	gonad; 2.6	0.862	0.283	0.328	++	+					
	test; 27.0	0.009	0.012	1.33	traces	traces					
(11 ♂)	intestine; 1.6	3.38	1.52	0.450	++	+					
	gonad; 5.3	4.48	0.528	0.118	++	+					
	test; 47.0	0.012	0.013	1.18	traces	traces					
HOLOTHUROIDEA											
Stichopus californicus											
(3)	whole; 127	0.013	0.010	0.769	+*	+	–	...	echinenone, pectenoxanthin.	–	–
(1)	whole; 58.0 (eviscerated)	0.015	0.021	1.40							

TABLE I—*Continued*

Animal (number analyzed)	Tissue; weight grams	Total epiphasic carotenoids mg./100 g.	Total hypophasic carotenoids mg./100 g.	Ratio; hypophasic/epiphasic	Carotenes	Xanthophylls	Xanthophyll esters	Acidic carotenoids or their precursors	Predominating *types* of carotenoid	Echinochromes	"Enterochlorophylls"
CARNIVORES											
ASTEROIDEA											
Astropecten californicus (17)	whole; 70.0	0.044	0.718	16.3	+*	+++	–	+	pectenoxanthin, astaxanthin.	–	–
Patiria miniata Color varieties (*a*) ochraceous buff, carmine, Indian purple (1)	whole; 176	0.028	0.434	15.5	+	+++	–	+	zeaxanthin, astaxanthin.	–	–
(*b*) scarlet, scarlet orange (1)	whole; 195	0.079	1.29	16.3	+	+++	–	+	zeaxanthin, astaxanthin.	–	–
Pisaster ochraceous Color varieties (*a*) Rufous (1)	2 rays+skin; 114	0.022	0.204	9.28	+	+++	–	+	zeaxanthin, astaxanthin, mytiloxanthin.		–
	pyloric caeca; 52	0.667	12.9	19.3	+	+++	–	+		–	+
(*b*) Chocolate (1)	2 rays+skin; 85	0.024	0.284	11.8	+	+++	–	+			–
	pyloric caeca; 13	0.455	9.01	19.8	+	+++	–	+		–	+
Pisaster giganteus (1)	2 rays+skin; 62	0.067	0.902	13.4	+	+++	traces	+	zeaxanthin, astaxanthin or metridene.	–	–
	pyloric caeca; 10.4	0.401	1.37	3.41	+	++	traces	+			+
OPHIUROIDEA											
Ophiopteris papillosa (4)	whole; 9.81	0.489	2.90	5.95	–	+++	+	?	taraxanthin, pectenoxanthin.	–	+
(53)	whole; 102	0.254	decomp.	(hypo.> epi.)							
Ophiothrix spiculata (35)	whole; 18	1.60	decomp.	(hypo.> epi.)	–	+++	+	+	taraxanthin; new xanthophylls.	–	+
Ophiothrix rudis (18)	whole; 8.8	1.40	decomp.	(hypo.> epi.)	–	+++	+	+	taraxanthin; new xanthophylls	–	+

* and echinenone.

Table II

Summary of Carotenoids in Echinoderms Studied

Animal	Epiphasic fraction (carotenes, esters)				Hypophasic fraction (xanthophylls, acidic carotenoids)			
	Chromatogram:				Chromatogram:			
	Total number of zones	Descending order of *principal* zones	Absorption maxima mμ	Corresponding known pigments	Total number of zones	Descending order of *principal* zones	Absorption maxima mμ	Corresponding known pigments
Dendraster excentricus	—	—	521; 484 (whole fraction)	β-carotene	—	—	518; 483 (whole fraction)	zeaxanthin
Strongylocentrotus franciscanus	6	II III IV	525; 488 520; 484 520; 484	echinenone β-carotene β-carotene	3	I (II) (III)	510; 479 511; 478 (whole fraction)	lutein lutein (isomers)
Strongylocentrotus purpuratus	5 to 9	I II III IV V	506; 478 — — 490 518; 483 510.5; 479.5	lutein *ester* echinenone β-carotene α-carotene	3 to 6	I II III	511; 481 511; 481 to 484 515; 480 to 484	petaloxanthin or antheraxanthin? zeaxanthin
Lytechinus pictus	3 to 7	I II III IV	490 519; 484 } 519; 486 } 507; 477	echinenone β-carotene α-carotene	4 to 5	I II III	512; 482 511; 479 509; 477	petaloxanthin or antheraxanthin lutein
Stichopus parvimensis	5	(combined extract)	521; 489	echinenone	4	(combined extract)	521; 489	pectenoxanthin?
Astropecten californicus	4	IV	519; 485	β-carotene	5	I II III	510; 481 505 (hot KOH →acid) 520; 489	antheraxanthin astacene or metridene pectenoxanthin
Patiria miniata	3 to 6	(combined extract)	—; 482 to 485	β-carotene (?)	4 to 6	II III	507 to 509 (hot KOH →acid) 517 to 521; 483 to 484	astaxanthin → astacene zeaxanthin

TABLE II—*Continued*

Animal	Epiphasic fraction (carotenes, esters)				Hypophasic fraction (xanthophylls, acidic carotenoids)			
	Chromatogram:		Absorption maxima mμ	Corresponding known pigments	Chromatogram:		Absorption maxima mμ	Corresponding known pigments
	Total number of zones	Descending order of *principal* zones			Total number of zones	Descending order of *principal* zones		
Pisaster ochraceous	—	—	— —	carotene(s)	5 to 7	I II III	502 to 505 (acidic) 506 to 510 (hot KOH →acid) 514 to 520; 483 to 485	mytiloxanthin astaxanthin → astacene zeaxanthin
Pisaster giganteus	6	(combined extract)	510; 483 519; 484	zeaxanthin *ester* β-carotene	5 to 7	I II III	516; 482 505; (495 in pyridine) (hot KOH →acid with same spectra) 513 to 518; 485 to 486	antheraxanthin metridene zeaxanthin
Ophiopteris papillosa	5	II III	497; — 505; — whole; 502; 471	esters taraxanthin *ester*	4	I II III IV	501; 470 503; 468 506 (not rendered acidic by hot KOH) 522; 489	taraxanthin a new xanthophyll? pectenoxanthin
Ophiothrix spiculata	11 or more	IX X XI	466 475 499; 466; 434	new acid on hydrolysis new acid on hydrolysis new xanthophyll *ester*	5	I II	495; 467 505	new xanthophyll new xanthophyll
Ophiothrix rudis	5	II V	505 460 whole; 497; 466 (before hydrolysis)	new acid on hydrolysis new acid on hydrolysis xanthophyll esters	more abundant than epiphasic fraction, but decomposed too rapidly for analysis			

like those encountered in the asteroids) but migrated quantitatively from this solvent to 90 per cent methanol in the partition test. It was adsorbed from petroleum ether solution at the top of a calcium carbonate column and was eluted therefrom with difficulty by methanol and acetic acid. Its solution in absolute ethanol gave a single band in the red at 663 mμ.

Ophiothrix spiculata: A yellow-green pigment remained in the diluted and salt-containing acetone extract from which all carotenoids had been transferred to petroleum ether. Insoluble in the latter solvent, even in the presence of dilute acids, this pigment was readily extracted with diethyl ether and transferred to absolute ethanol, in which it showed a single absorption band at 663 mμ.

Ophiothrix rudis yielded small quantities of a green pigment similar in solubility properties to that recovered from *O. spiculata.*

MacMunn's green pigments extracted from the radial caeca of *Solaster* and *Uraster* displayed several spectral bands in alcohol solution, one in the vicinity of a single band found in the red by ourselves, and the others in additional regions similar to those shown for chlorophyll. He considered that such green pigments were breakdown products of chlorophyll (MacMunn, 1886).

We have at present no new suggestions to add to MacMunn's conclusions. While the spectra of our green pigments were far simpler than those of MacMunn or than those of chlorophyll, it is likely that these pigments in the digestive organs of carnivores represent porphyrins from an *original* source of chlorophyll.

Discussion

The major results obtained in this study are summarized in Table I. The animals are grouped according to their food habits. The echinoids feed on kelp, on detritus that is predominantly of vegetable orgin, or upon fixed algae, while the holothurians are bottom dwellers that subsist on the latter two classes of food. The asteroids feed exclusively on animal matter, especially mollusks, while the ophiuroids feed mainly on very small particulate animal matter.

A few calculations based upon information given in Table I reveal some interesting qualitative and quantitative differences between the carotenoids of herbivores and those of carnivores. For example, a total weight of 847 grams of tissue from herbivorous forms yielded some 4.27 mg. of carotenoid pigment (β-carotene equivalents), or an average concentration of about 0.50 mg. per 100 grams of fresh tissue. In the carnivores, on the other hand, 916 grams of tissue yielded a measurable quantity of some 14.61 mg. of carotenoids (β-carotene equivalents), giving an average concentration of about 1.60 mg. per 100 grams of fresh tissue. The average ratios of hypophasic to epiphasic pigments were, in the herbivores, 0.86, and in the carnivores, at least 13. Furthermore, account is to be taken of the fact that the hypophasic pigments in the ophiuroids were, as in the other carnivores, far in excess of the epiphasic ones, but that, in three out of four analyses, the color of the hypophasic fraction bleached before quantitative estimations could be made. If we assume that the hypophasic pigments in the second catch of *Ophiopteris papillosa* exceeded the epiphasic fraction by the same ratio as was found in the first catch (i.e. about 6) and that this ratio could be applied also to the other two ophiuroid species, *Ophiothrix spiculata* and *O. rudis,* we then arrive at a figure of 2 mg. of carotenoids per 100 grams of fresh carnivore tissue.

Among the carnivores, the ophiuroids contained oxygenated carotenoids exclusively, the epiphasic fractions yielding no carotenes, but only esterified xanthophylls and acidic carotenoids. The asteroids likewise showed a great preponderance of oxygenated over hydrocarbon carotenoids. *Pisaster giganteus* contained an epiphasic esterified xanthophyll which behaved like zeaxanthin. In the herbivores, the echinoids too contained some xanthophyllic pigments without exception, and most of them yielded an oxygenated carotenoid, echinenone, in the epiphasic fraction. One of three catches of *Strongylocentrotus purpuratus* yielded an esterified xanthophyll resembling lutein.

In summary, carnivorous species contained three to four-fold the quantity of carotenoid pigments found in herbivores. The oxygenated type of carotenoid, including xanthophylls and acidic compounds, preponderated vastly over carotenes [2] in carnivorous species. However, the herbivores, with the exception of the urchin *Strongylocentrotus purpuratus* which showed a slight excess of xanthophylls over carotenes, possessed predominating quantities of the latter type of pigment. Finally, the non-hydrocarbon carotenoids stored in carnivorous animals were more heavily oxygenated than those in the herbivores, the former yielding numerous carotenoid acids, as well as taraxanthin and similar xanthophylls, none of which were encountered in any of the herbivores.

The apparently greater capacity of the carnivorous, as compared with the herbivorous echinoderms, to assimilate or store carotenoids, with perhaps a certain degree of oxidative modification, may be associ-

[2] It should be understood that the term "carotenes" as used here means those epiphasic pigments resistant to alkaline hydrolysis, and hence may include such mono-hydroxy xanthophylls as cryptoxanthin and the ketone echinenone. The possible presence of cryptoxanthin was usually eliminated, however, by employing in the partition test 95 per cent methanol, in which this pigment is preferentially soluble.

ated with less metabolic utilization of the oxidized pigments than of the carotenes. At present nothing seems to be known either of the requirements of marine invertebrates for vitamin A or its precursors, or of specific rôles played by carotenoids in lower phyla.

The carotenoid acids are of especial interest and may be classified into three types. *In vivo* some are neutral xanthophylls but treatment with alcoholic alkali produces molecular rearrangements and oxidations which lead to the formation of acids (*cf.* astaxanthin). This type was found only in the asteroids but was present in all such specimens examined. The second type is acidic as it is extracted from the tissues. It was found only in *Pisaster,* and since it resembles strongly the mytiloxanthin described by Scheer (1940) from the California mussel, it is likely that *Pisaster* derives this pigment from the mollusk, upon which it feeds extensively. Finally, acids may occur *in vivo* as esters, in which case they are epiphasic before hydrolysis, their acidic properties becoming evident after treatment with alcoholic potash. These esters were found only in ophiurans and gave on hydrolysis a new type of pigment, which is acidic, and has a single absorption maximum at 460, 466 or 475 mμ. In addition, an acid-yielding ester of the more conventional type with a single absorption maximum at 505 mμ appeared.

As in the lobster, carotenoid acids were found to be combined with protein in one case. *Pisaster giganteus* contains purple, blue and yellow chromoproteins in the skin, the purple and blue patches of which yielded an unesterified carotenoid, which formed an acid on treatment with alkali. This pigment resembled the anemone pigment metridene (Fox and Pantin, 1941).

Table II shows some similarities between certain of the echinoderm carotenoids and known pigments. A commonly occurring one showed a spectrum like that of β-carotene or zeaxanthin; pigments in this group were found in all classes except the Ophiuroidea, which yielded instead pigments with spectra like taraxanthin, not found in the other classes. Spectra like that of α-carotene or lutein were not frequently encountered, except among the echinoids, wherein both carotenes and xanthophylls with this type of spectrum were found. Pigments resembling echinenone have been mentioned above, notably in the epiphase of echinoid extracts (Lederer, 1938). The unchromatographed extracts, both epiphasic and hypophasic, of *Stichopus* also showed the echinenone type of spectrum; again the alkali-resistant epiphase and the hypophase of one of the carnivores, i.e., *Astropecten,* as well as certain hypophasic pigments (rare xanthophylls such as pectenoxanthin) in other groups, manifested the same absorption maxima.

Where individual tissues were studied, the highest values were found in the digestive tract, excepting in the testes of *Lytechinus;* certain asteroid skins would also show relatively high values were they readily separable from the colorless skeleton.

The few correlations which could be observed between sex and carotenoid content in tissues were limited to three of the echinoid species. In *Dendraster,* males contained about twice the concentration of epiphasic and thrice the hypophasic pigments found in females. Estimations of pigments in individual tissues were not made. In *Strongylocentrotus purpuratus,* the skin of each sex yielded only traces of carotenoids. In the intestine, epiphasic pigments were plentiful and of the same order of concentration in both sexes; the same was true of the hypophasic compounds. However, carotenoids in ovaries exceeded those in testes by three-fold, and were entirely epiphasic, xanthophylls being absent. In *Lytechinus,* whole males yielded somewhat more of each class of carotenoid than did whole females. This was due to a five-fold excess of epiphasic and nearly a two-fold excess of hypophasic carotenoids in testicular over ovarian tissues. Intestines, considerably lighter in mass than gonad material, were also fairly rich in pigment, females yielding nearly a third again as much epiphasic and almost three times as much hypophasic pigment as males. The skin of *Lytechinus,* like that of *Strongylocentrotus,* contained only traces of carotenoids.

Females of *Strongylocentrotus* and *Lytechinus* contained some 25 and 27 per cent, respectively, of their carotenoids in the ovaries, whereas corresponding males mobilized approximately 17 and 77 per cent, respectively, to their testes, although the sperm itself was not colored.

The carotenoid distribution ratio between intestine and ovary is doubtless subject to variation with discharge of ripe eggs. There still remains the striking mobilization of carotenoids to testicular tissues, especially in *Lytechinus.* Also remarkable was the complete absence of xanthophylls from the gonads of both sexes of *Strongylocentrotus.*

Three types of non-carotenoid pigment were encountered in this study. Insoluble pigments, presumably of the melanin type, appeared in *Stichopus* and *Pisaster ochraceus,* but were not studied. Green pigments, the "enterochlorophylls" of MacMunn, were obtained from certain of the carnivores, but were never found in starved herbivores. These pigments invariably had absorption maxima at 661 and 663 mμ in absolute ethanol. From all the echinoids, but from no others, much red to purple pigment of the echinchrome type was extracted. It was found as calcium salt in the testes and spines, and as purple (basic) or red (acidic) irregular bodies dispersed in the tissues of ectoderm, endoderm, intestine, and in some cases, in the gonads. Although quantitative studies were not made, echinochrome was clearly most abundant in *Strongylocentrotus,* quite abundant in *Dendraster,* and present in relatively small amounts in *Lytechinus.*

In conclusion it may be reiterated that the echinoderms represent a structurally and ecologically diverse group of much interest for further investigations of a comparative biochemical nature, especially regarding the metabolism of colored compounds.

Summary

1. The pigments of echinoderms, belonging to twelve species, nine genera and four classes, have been studied qualitatively and in part quantitatively, with the aid of certain standard methods.

2. Carnivorous species contained more carotenoids in the aggregate than did herbivores, by some three or four-fold.

3. Among the echinoids, *Dendraster* and *Lytechinus* males yielded more total carotenoids than did females, while in *Strongylocentrotus purpuratus,* the concentrations in each sex were similar. Females of *Strongylocentrotus* and *Lytechinus* contained about a fourth of their carotenoids in the ovaries, whereas corresponding males mobilized about one-sixth and three-quarters, respectively, to their testes.

4. Oxygenated carotenoids, including xanthophylls and acidic compounds, preponderated vastly over the hydrocarbon type (carotenes) in carnivores, while in most herbivores epiphasic pigments, including carotenes and echinenone, showed some degree of predominance.

5. The presence of the ketonic carotenoid echinenone was indicated in most of the echinoids; its presence was also regarded as likely in another member of the herbivore-omnivore group, i.e., the cucumber *Stichopus,* and in one carnivore, *Astropecten.*

6. Carotenoid acids, or compounds which yield carotenoid acids on treatment with alkali, were found only in carnivorous species. A new type of epiphasic pigment, with a single absorption maximum in the violet at values of from 460 to 475 $m\mu$, yielding an acid on hydrolysis, was found in the ophiuroids.

7. Xanthophyll esters were found consistently in the Ophiuroidea and in one of the Asteroidea, *Pisaster giganteus.* In the ophiuroids, they were of the heavily oxygenated type, and replaced carotenes, which were completely lacking. One of three catches of *Strongylocentrotus purpuratus* yielded some esterified lutein-like xanthophyll.

8. The occurrence of echinochromes, found only in the echinoids, and of green pigments, " enterochlorophylls," found in the intestines of carnivores, is discussed.

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